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APMicroDB: A microsatellite database of *Acyrthosiphon pisum*

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ABSTRACT

Pea aphids represent a complex genetic system that could be used for QTL analysis, genetic diversity and population genetics studies. Here, we described the development of first microsatellite repeat database of the pea aphid (APMicroDB), accessible at "http://deepaklab.com/aphidmicrodb". We identified 3,40,233 SSRs using MIcroSAtellite (MISA) tool that was distributed in 14,067 (out of 23,924) scaffold of the pea aphid. We observed 89.53% simple repeats of which 73.41% were mono-nucleotide, followed by di-nucleotide repeats. This database stored information about the repeats kind, GC content, motif type (mono - hexa), genomic location etc. We have also incorporated the primer information derived from Primer3 software of the 250bp flanking region of the identified marker. Blast tool is also provided for searching the user query sequence for identified marker and their primers. This work has an immense use for scientific community working in the field of agricultural pest management, QTL mapping, and host-pathogen interaction analysis.

1. Introduction

Simple Sequence Repeats (SSRs) also known as Microsatellites, are the extensively dispersed short tandem repeat units harbor substantial length variation [1,2]. A major proportion of eukaryotic genomes (up to 4%) are composed of these markers. Despite their presence in both coding and non-coding region, high abundance was only observed in the non-coding region of the genome [3,4]. Previous studies suggested that short tandem repeats (STRs) are under the selective pressure that played an important role in genome structure and evolution [5–7].

SSRs offers several advantages such as their distribution, specificity, and reproducibility, therefore, they were extensively employed in population genetics [8,9], genetic diversity [10–13] and evolution [14,15]. Based on the origin, SSRs has been classified into two types: 1) genomic SSRs (that derived from genome), and 2) EST-SSRs (that comes from expressed sequence tags) [10,14]. EST-based SSRs were originated from transcribed region which is more conserved as compared to genomic SSRs [16,17]. Therefore, genomic SSRs are highly polymorphic and fitted for genetic diversity studies within a particular species.

The present study is focused on the identification of SSRs from the genome of *A. pisum.* Pea aphids (*Acyrthosiphon pisum*) are the phloem-feeding insects having several advantages over other aphid species [18]. Association of pea aphid with more than 20 legume genera represents their host race specific evolution. Each race is more or less specialized and genetically differentiated from other host races [19,20]. To reveals the host-pathogen relationship, it is important to understand the genomic architecture of aphid genome. Hence, the international aphid genome consortium first time reported the draft genome of the pea aphid of size 464 Mb. Initially, ~3.13 million reads were assembled into 72,844 contigs using Atlas assembly pipeline. However, in the second version, the number of contigs was reduced to 60,596 with the N50 length of around 28 kb. Previously, only few studies have been reported to experimentally characterize the microsatellite markers in pea aphid [21–23]. However, the wet-lab characterization is very tedious and time-consuming job. Therefore, researchers paved the attention for *in silico* identification of SSRs in the aphid genome [2,24]. For *e.g.* Behura et al. reported 1,69,601 and 4283 microsatellite repeats in whole genome and coding region of *A. pisum* respectively. Based on the identified SSRs, few insect specific databases such as InSatDb, EuMicrosatdb etc. has been developed in the past [25,26]. Best of the author knowledge, no publicly accessible database of SSRs has been reported for the pea aphid. Owing to the importance of microsatellite, and pea aphid as model insect species, the foremost purpose of this manuscript is to discover the abundance and distribution of SSRs in the pea aphid genome.

2. Database development

2.1. Database construction and architecture

We have downloaded the pea aphid genome v2.0 from the NCBI database in FASTA format [27]. The complete genome was scaffold-wise scan for the occurrence of microsatellite repeats using MIcroSAtellite (MISA) tool (http://pgrc.ipk-gatersleben.de/misa/). We used the PRIMER3 software to predict the primer of the identified microsatellite markers [28]. For this, we extracted a flanking region of 250 bp of the

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Table 1
Overall distribution of SSRs and their percentage in pea aphid genome.

SSR type	Count	Percentage
Simple	3,04,595	89.53%
Compound	34,196	10.05
Complex	1442	0.42
Total	3,40,233	100%

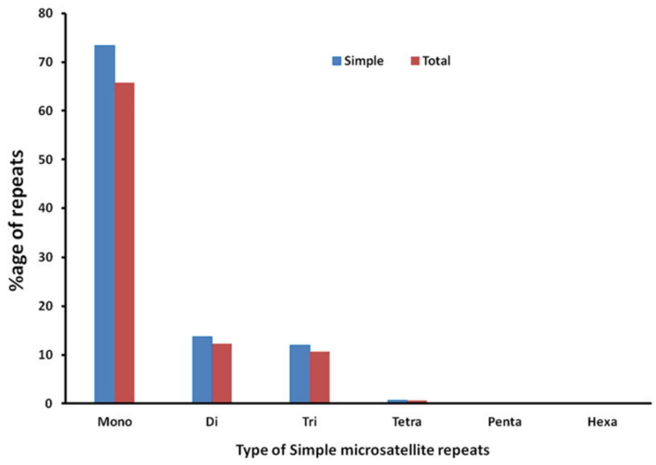


Fig. 1. Histogram plot of SSRs with the type of repeats in the x-axis and their percentage in the y-axis.

repeats on both sides using bedtools [29]. The custom PERL scripts were used to process the MISA output in CSV format. Finally, the file was uploaded into MySQL database. The front-end of the database was developed using HTML, PHP language, and JAVA scripts.

2.2. Genome analysis

We analyzed the distribution of STRs across the scaffold and observed that simple microsatellite repeats represents 89.53% of the total STRs (Table 1). We also plotted the different motif repeats from mono- hexa to show their relative abundance in pea aphid genome. As evident from Fig. 1 and Table-S1, Mononucleotide type repeats (73.41%) was most abundant as compared to other types [30,31]. However, hexanucleotide repeats (0.03%) was the least ones (suppl-1.docx, Table-S1). Our analysis also supported the Katti et al. analysis that trinucleotide repeats have a maximum length 441 bp followed by dinucleotides (suppl-1.docx, Table-S1) [32]. We also observed that STRs of length up to 15 bp represents the major proportion in the genome followed by length 16–20 (Fig. 2). However, the motif of length 46–50 bp was represented by only 0.13% (Fig. 2, Table-S2).

2.3. STR validation

Previously, Kurokawa et al. reported six microsatellite markers in pea aphid using experimental approach [21]. In the same year, Caillaud et al. reported fifteen markers from pea aphids [22]. In order to validate this, we used the FASTA sequence of reported marker and search in our database using blast tool. We observed that 76% of the markers were partially or completely matched with our database (Table 2). Out of the 15 markers, we found six were exactly matched, and seven markers matched with repeat kind but their copy number has been changed. This might be because the assembly of pea aphid genome is only available at preliminary scaffold level but not at the chromosome level.

3. Utility

3.1. Search

We provided the scaffold wise search option for STRs along with the marker properties such as the type of motif, repeat kind etc. Furthermore, we have also given the advanced search option to filter the results based on the scaffold region, copy number of the marker, and GC content. This will be helpful to the user interested in locating the marker in the given genomic region of the genome, which may be coding or non-coding. The search result is shown in a well-organized tabular format with an additional button for extracting primer information of a particular SSR (Fig. 3). On clicking the show primer button, users will

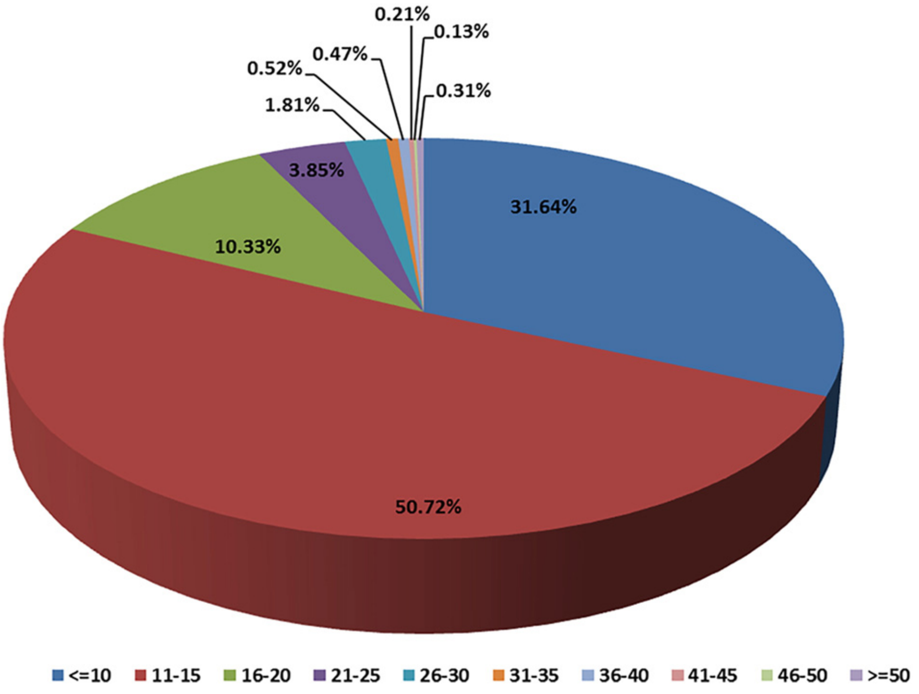


Fig. 2. Pie chart showing the percent distribution of microsatellite repeats within different length ranges.

Table 2
Validation of previously identified STRs with APMicroDB.

GenBank	Scaffold no.	Reported [21,22]	APMicroDB
AY528722	NW_003383558	(TC)12	(TC)10
AY528723	NW_003383960	(CT)12	(CT)10g(TC)7
AY528724	NW_003383777	(GA)11	(GA)10
AY528725	NW_003383909	(AG)20	(AG)14
AY528726	NW_003383570	(CT)10	(CT)14
AY528727	NW_003383545	(AG)7AT(AG)18	(AG)7at(AG)16
AY528728	NW_003383554	(CA)4T(AC)4	Not found
AY528729	NW_003384067	(GT)6	(GT)6
AY528730	NW_003399631	(TG)2TA(TG)8	(TG)8
AY528731	NW_003383549	(GCT)8	(GCT)8
AY528732	NW_003384067	(AC)5GAAT(AC)4	Not found
AY528733	NW_003383549	(AGC)8	(AGC)8
AY528734	NW_003384434	(CA)10	(CA)10
AY528735	NW_003383752	(CA)16	(CA)16
AY528736	NW_003384150	(CA)7 … (TA)3T(CA)3	(TG)7-
AB162918	NW_003383507	(ATA)5	Not found
AB162919	NW_003383919	(CG)5 … (TTA)7	(T)12gggggggaagggtccggtgtaaaaattgaaagtaaaaaacgaattcaaatacaaaaaacacaggtacaatctcgtatag(TAA)7
AB162920	NW_003385021	(GA)11	(GA)19
AB162921	NW_003383764	(AT)9	Not found
AB162922	NW_003383818	(AC)7 … (AC)5	Not found
AB162923	NW_003383520	(TG)8	(TG)6

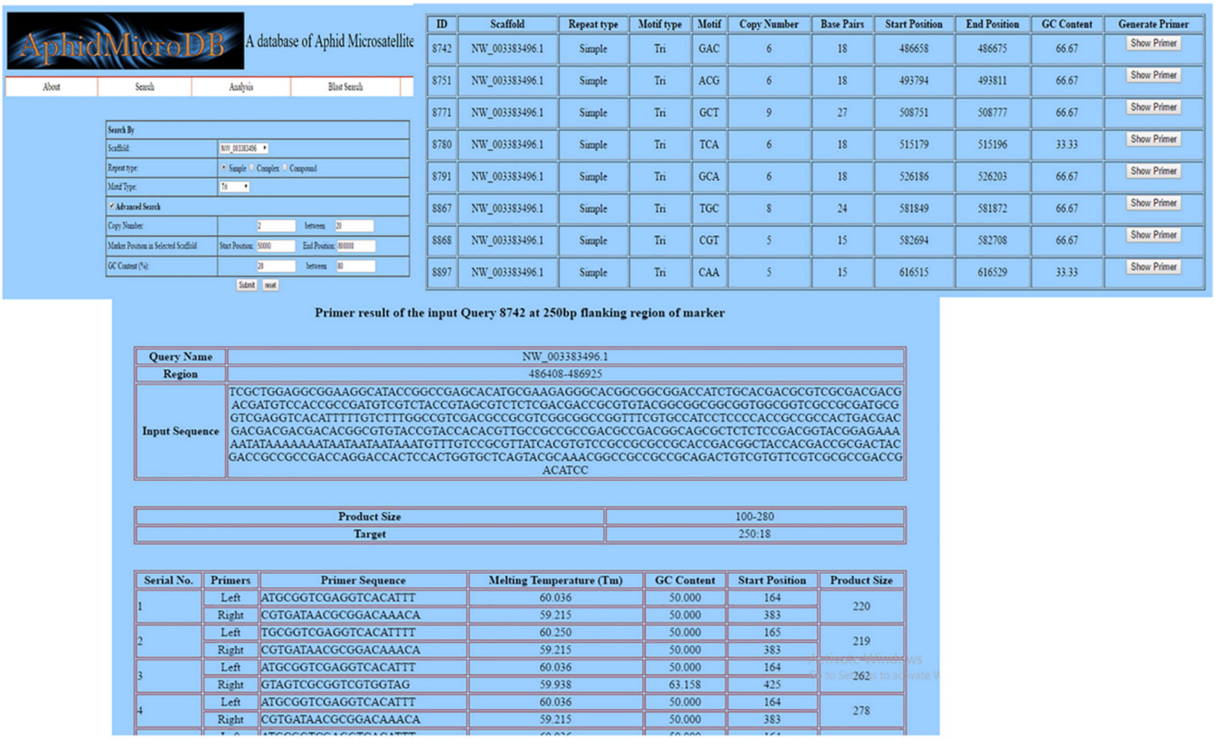
get the information about the primers (250 bp flanking region of marker) and their properties.

3.2. Web tool

A customized BLAST tool is implemented in this database for similarity search. The user input query sequence will be searched against the database of repeats containing flanking region. A user-friendly search option for e-value cut off, query coverage and a number of hits to be displayed is provided in the blast search. The identified hit is further linked with the primer information of the identified hits (Fig. 4).

4. Discussion

Here, we reported the mining of 3,40,233 microsatellite markers, which is almost double that are reported by Behura and Severson [24]. The percentage of mono- was higher followed by di-, tri-, tetra, penta, and hexa-nucleotide repeats respectively. A similar trend was observed by Sharma et al. supporting the fact that an increase in repeat length is proportional with the decrease in repeat numbers [31]. The distribution of repeat length showed a good coverage in the range of 11–15 bp long repeats. However, low coverage (0.13%) was observed in the case of repeats of length 46–50 bp. In 2001, Katti et al. observed that tri-nucleotide repeat seems to be much longer as compared to other repeats in



Search By

Scaffold:	NW_003383496
Repeat type:	● Simple ○ Complex ○ Compound
Motif Type:	Tri
☑ Advanced Search	
Copy Number:	2 between 20
Marker Position in Selected Scaffold	Start Position: 50000 End Position: 800000
GC Content (%):	20 between 80

ID	Scaffold	Repeat type	Motif type	Motif	Copy Number	Base Pairs	Start Position	End Position	GC Content	Generate Primer
8742	NW_003383496.1	Simple	Tri	GAC	6	18	486658	486675	66.67	Show Primer
8751	NW_003383496.1	Simple	Tri	ACG	6	18	493794	493811	66.67	Show Primer
8771	NW_003383496.1	Simple	Tri	GCT	9	27	508751	508777	66.67	Show Primer
8780	NW_003383496.1	Simple	Tri	TCA	6	18	515179	515196	33.33	Show Primer
8791	NW_003383496.1	Simple	Tri	GCA	6	18	526186	526203	66.67	Show Primer
8867	NW_003383496.1	Simple	Tri	TGC	8	24	581849	581872	66.67	Show Primer
8868	NW_003383496.1	Simple	Tri	CGT	5	15	582694	582708	66.67	Show Primer
8897	NW_003383496.1	Simple	Tri	CAA	5	15	616515	616529	33.33	Show Primer

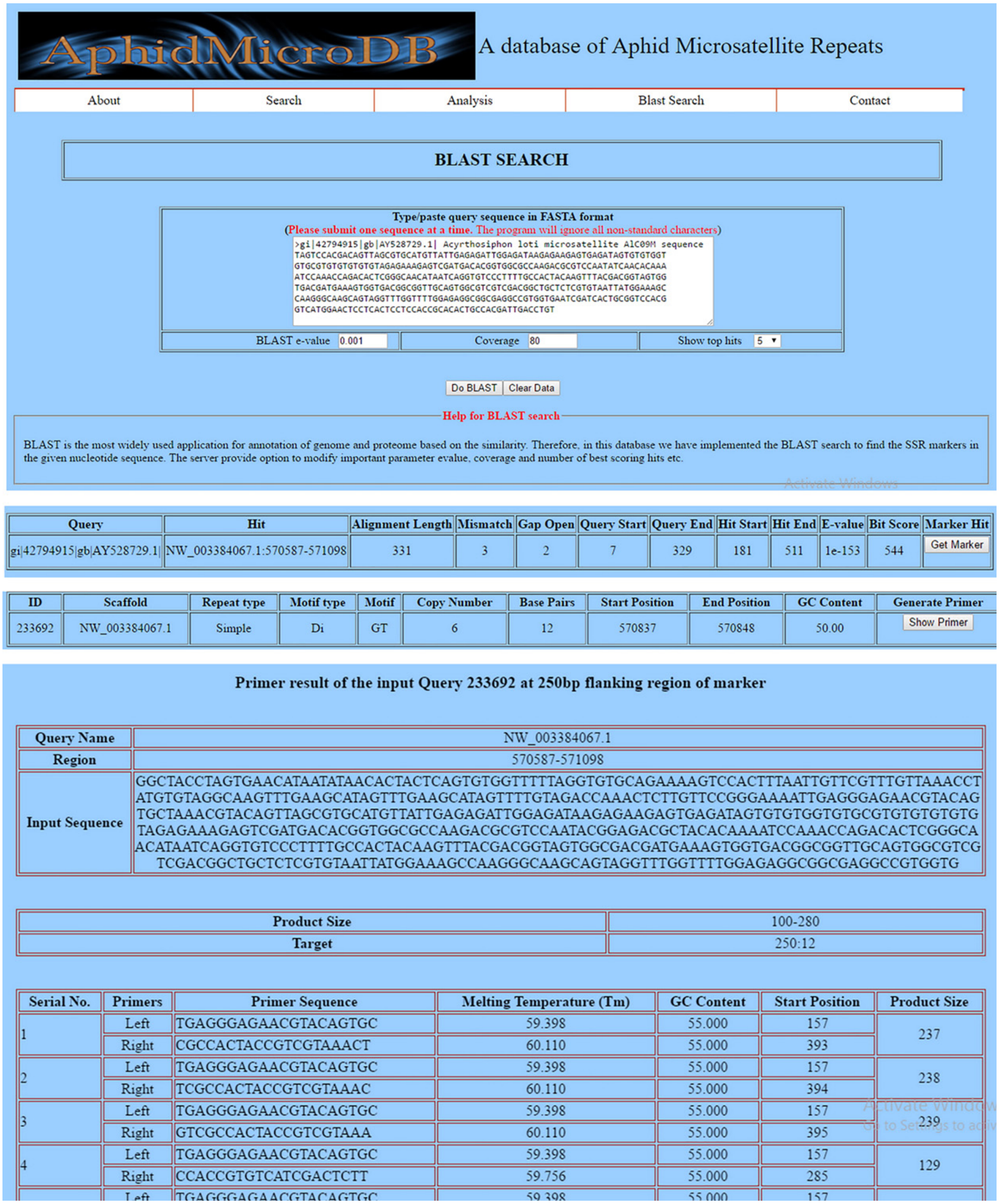
Primer result of the input Query 8742 at 250bp flanking region of marker

Query Name	NW_003383496.1
Region	486408-486925
Input Sequence	TCGCTGGAGGCGGAAGGCATACCGGCCGAGCACATGCGAAGAGGGCACGGCGGCGGACCATCTGCACGACGCGTCGCGACGACG ACGATGTCCACCGCCGATGTCGTCTACCGTAGCGTCTCTCGACGACCGCGTGTACGGCGGCGGCGGTGGCGGTCGCCGCGATGCG GTCGAGGTCACATTTTTGTCTTTGGCCGTCGACGCCGCGTCGGCGGCCGGTTTCGTGCCATCCTCCCCACCGCCGCCACTGACGAC GACGACGACGACACGGCGTGTACCGTACCACACGTTGCCGCCGCCGACGCCGACGGCAGCGCTCTCTCCGACGGTACGGAGAAA AATATAAAAAAATAATAATAATAAATGTTTGTCCGCGTTATCACGTGTCCGCCGCGCCGCACCGACGGCTACCACGACCGCGACTAC GACCGCCGCCGACCAGGACCACTCCACTGGTGCTCAGTACGCAAACGGCCGCCGCCGCAGACTGTCGTGTTCGTCGCGCCGACCG ACATCC

Product Size	100-280
Target	250:18

Serial No.	Primers	Primer Sequence	Melting Temperature (Tm)	GC Content	Start Position	Product Size
1	Left	ATGCGGTCGAGGTCACATTT	60.036	50.000	164	220
	Right	CGTGATAACGCGGACAAACA	59.215	50.000	383	
2	Left	TGCGGTCGAGGTCACATTTT	60.250	50.000	165	219
	Right	CGTGATAACGCGGACAAACA	59.215	50.000	383	
3	Left	ATGCGGTCGAGGTCACATTT	60.036	50.000	164	262
	Right	GTAGTCGCGGTCGTGGTAG	59.938	63.158	425	
4	Left	ATGCGGTCGAGGTCACATTT	60.036	50.000	164	278
	Right	CGTGATAACGCGGACAAACA	59.215	50.000	383	

Fig. 3. Showing the database search page and its results along with primer information.



BLAST SEARCH

Type/paste query sequence in FASTA format
(Please submit one sequence at a time. The program will ignore all non-standard characters)

```
>gi|42794915|gb|AY528729.1| Acyrthosiphon loti microsatellite AlC09M sequence
TAGTCCACGACAGTTAGCGTGCATGTTATTGAGAGATTGGAGATAAGAGAAGAGTGAGATAGTGTGTGGT
GTGCGTGTGTGTGTGTAGAGAAAGAGTCGATGACACGGTGGCGCCAAGACGCGTCCAATATCAACACAAA
ATCCAAACCAGACACTCGGGCAACATAATCAGGTGTCCCTTTTGCCACTACAAGTTTACGACGGTAGTGG
TGACGATGAAAGTGGTGACGGCGGTTGCAGTGGCGTCGTCGACGGCTGCTCTCGTGTAATTATGGAAAGC
CAAGGGCAAGCAGTAGGTTTGGTTTTGGAGAGGCGGCGAGGCCGTGGTGAATCGATCACTGCGGTCCACG
GTCATGGAACTCCTCACTCCTCCACCGCACACTGCCACGATTGACCTGT
```

BLAST e-value 0.001 | Coverage 80 | Show top hits 5

Help for BLAST search

BLAST is the most widely used application for annotation of genome and proteome based on the similarity. Therefore, in this database we have implemented the BLAST search to find the SSR markers in the given nucleotide sequence. The server provide option to modify important parameter evalue, coverage and number of best scoring hits etc.

Query	Hit	Alignment Length	Mismatch	Gap Open	Query Start	Query End	Hit Start	Hit End	E-value	Bit Score	Marker Hit
gi|42794915|gb|AY528729.1|	NW_003384067.1:570587-571098	331	3	2	7	329	181	511	1e-153	544	Get Marker

ID	Scaffold	Repeat type	Motif type	Motif	Copy Number	Base Pairs	Start Position	End Position	GC Content	Generate Primer
233692	NW_003384067.1	Simple	Di	GT	6	12	570837	570848	50.00	Show Primer

Primer result of the input Query 233692 at 250bp flanking region of marker

Query Name	NW_003384067.1
Region	570587-571098
Input Sequence	GGCTACCTAGTGAACATAATATAACACTACTCAGTGTGGTTTTTAGGTGTGCAGAAAAGTCCACTTTAATTGTTCGTTTGTTAAACCT ATGTGTAGGCAAGTTTGAAGCATAGTTTGAAGCATAGTTTTGTAGACCAAACTCTTGTTCCGGGAAAATTGAGGGAGAACGTACAG TGCTAAACGTACAGTTAGCGTGCATGTTATTGAGAGATTGGAGATAAGAGAAGAGTGAGATAGTGTGTGGTGTGCGTGTGTGTGTG TAGAGAAAGAGTCGATGACACGGTGGCGCCAAGACGCGTCCAATACGGAGACGCTACACAAAATCCAAACCAGACACTCGGGCA ACATAATCAGGTGTCCCTTTTGCCACTACAAGTTTACGACGGTAGTGGCGACGATGAAAGTGGTGACGGCGGTTGCAGTGGCGTCG TCGACGGCTGCTCTCGTGTAATTATGGAAAGCCAAGGGCAAGCAGTAGGTTTGGTTTTGGAGAGGCGGCGAGGCCGTGGTG

Product Size	100-280
Target	250:12

Serial No.	Primers	Primer Sequence	Melting Temperature (Tm)	GC Content	Start Position	Product Size
1	Left	TGAGGGAGAACGTACAGTGC	59.398	55.000	157	237
	Right	CGCCACTACCGTCGTAAACT	60.110	55.000	393	
2	Left	TGAGGGAGAACGTACAGTGC	59.398	55.000	157	238
	Right	TCGCCACTACCGTCGTAAAC	60.110	55.000	394	
3	Left	TGAGGGAGAACGTACAGTGC	59.398	55.000	157	239
	Right	GTCGCCACTACCGTCGTAAA	60.110	55.000	395	
4	Left	TGAGGGAGAACGTACAGTGC	59.398	55.000	157	129
	Right	CCACCGTGTCATCGACTCTT	59.756	55.000	285	
	Left	TGAGGGAGAACGTACAGTGC	59.398	55.000	157	

Fig. 4. The overall flow of user Blast query, and its link to database and primers.

Drosophila [32]. This is highly correlated with our study of pea aphid that belongs to the same phylum. A significant correlation with the previously identified marker suggests the application of this database. Despite the improvement in pea aphid assembly from version1.0 to version 2.0 still the assembly existed at the scaffold level. This indicates a gap in the knowledge of SSR markers in pea aphids and suggested that there must be a much more SSRs marker that could only be resolved only at the chromosome level.

5. Data maintenance

APMicroDB will be regularly maintained by our team. We will welcome any scientific suggestion from the readers via. 'Contact' link on the database. In future, we will upgrade the database whenever the new assembly from different strain/race of pea aphid will be reported. The update will be helpful in study species-specific primer and establish an evolutionary relationship.

6. Conclusion

STRs are the most extensively studied marker having wide application in genetic diversity, evolution, and genome mapping. Despite the great importance of microsatellite makers, no database exists to store and compiles the genome-wide information of SSR markers from pea aphid. Therefore, in the present work, an effort has been made to develop first whole genome based SSRs database of pea aphid that will be useful in phylogenetic analysis, and evolutionary insight on pea aphid.

Competing interests

The authors declare that they have no competing interests.

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Abbreviations

SSRs	Simple Sequence Repeats
STR	Short tandem repeat
MISA	MIcroSAtellite
bp	base pair
HTML	Hyper Text Markup Language
PHP	Hypertext Preprocessor

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2017.03.014.

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